

## Occurrence of Sclerotium Rot in *Allium tuberosum* Caused by *Sclerotium rolfsii* in Korea

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In this study, we characterized sporadically occurring sclerotium rot caused by *Sclerotium rolfsii* in Chinese chive (*Allium tuberosum* Roth.) in farm fields in Sacheon, Korea. The initial symptom of the disease was water-soaked, which progressed to rotting, wilting, blighting, and eventually death. Further, mycelial mats spread over the lesions near the soil line, and sclerotia formed on the scaly stem and leaves. The sclerotia were globoid, 1~3 mm, and white to brown. The optimum temperature for growth and sclerotia formation on potato dextrose agar (PDA) was 30°C. The diameter of the hyphae ranged from 4 to 8 µm. Clamp connection was observed on PDA medium after 5 days of incubation. Based on the mycological characteristics, internal transcribed spacer sequence analysis, and pathogenicity test, the causal agent was identified as *Sclerotium rolfsii* Saccardo. This is the first report of sclerotium rot in Chinese chive caused by *S. rolfsii* in Korea.

**KEYWORDS :** Chinese chive, *Sclerotium rolfsii*, Sclerotium rot

This study investigated a sporadically occurring scaly stem and leaf rot disease caused by *Sclerotium rolfsii* in Chinese chive (*Allium tuberosum* Roth.) in farm fields in Onjeong-ri, Yonghyeon-myeon, Sacheon, Korea in 2010.

In August, the scaly stems and leaves under the canopy of Chinese chive after planting are exposed to warm temperatures and high humidity conditions, which favor disease development of mulching cultivation in vinyl houses (Fig. 1A). We observed that the sporadically occurring disease developed mainly on scaly stems near the soil line. Infected plants gradually withered, a white mycelial mat appeared, and numerous sclerotia were produced on scaly stems surfaces near the soil line (Fig. 1B). The heavily infected scaly stems and leaves became rotted, blighted, and the whole plant eventually died.

Fifty samples of scaly stems and leaves of Chinese chive caused by *Sclerotium rolfsii* were cut, and after isolation, the pathogenic fungus was grown on potato dextrose agar (PDA). Detailed microscopic examination of a representative specimen was performed by scanning electron microscopy (LEO 1420VP; LEO Electron Microscopy Ltd., Cambridge, UK) and light microscopy (Axioplan 2; Carl Zeiss, Jena, Germany). The optimal growth temperature on PDA was 30°C. Aerial mycelia usually formed many narrow hyphal strands 4~8 µm wide. The white mycelium formed a typical clamp connection

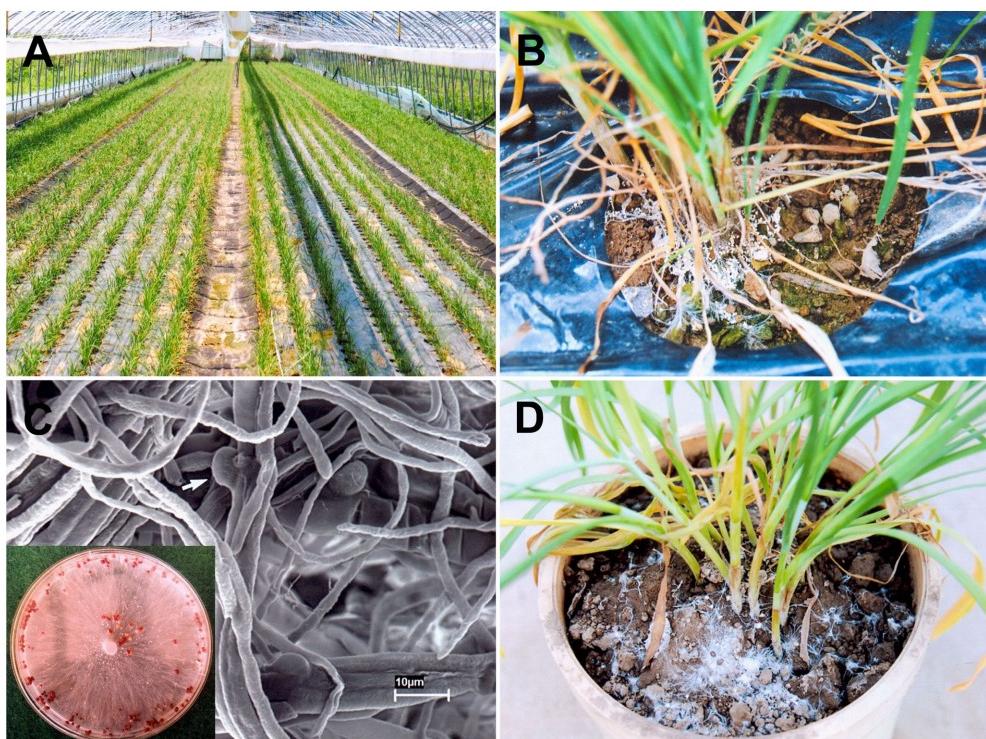
structure after 5 days of growth at optimum temperature (Fig. 1C). The sclerotia were white at first, after which they gradually turned dark brown in color, and were 1~3 mm in diameter. The maximum number of sclerotia was produced at 25~30°C. Small globoid sclerotia formed abundantly on PDA after 18 days of mycelial growth (Table 1, Fig. 1C) [1].

To test pathogenicity, inoculum was prepared as previously described [2]. Briefly, the mycelial mat of the test fungus grown on PDA for 7 days was harvested and mixed thoroughly with sterilized soil. The soil mixture was used as an inoculum; 200 g of soil inoculum was placed on top of Wagner's pots, after which Chinese chive seedlings were transplanted. The inoculated pots were kept separately in a green house and observed for disease symptoms. After 12 days of inoculation, the same disease symptoms were observed, and the fungus was re-isolated from the plants inoculated artificially (Fig. 1D).

To confirm the identity of the fungus, we amplified and sequenced an internal transcribed spacer (ITS) rDNA region of the isolate using the primers ITS1 and ITS4, as described by White *et al.* [3]. The resulting 685 bp sequence was deposited in GenBank (accession No. JF966208). Comparison with other sequences available in the GenBank database revealed that the ITS sequence shared 100% similarity with sequences of *S. rolfsii* (GenBank accession

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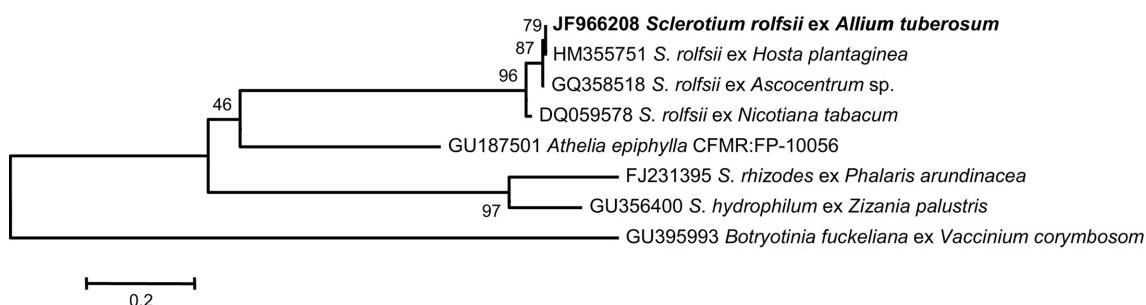
**Fig. 1.** Symptoms of sclerotium rot in Chinese chive (*Allium tuberosum* Roth.) and mycological characteristics of the pathogenic fungus *Sclerotium rolfsii*. A, Farmers vinyl-house culture of Chinese chive; B, Typical field symptoms on scaly stems near the soil line; C, Scanning electron micrographic image of clamp connection (arrow). Mycelial mat and sclerotia grown on potato dextrose agar after 18 days of incubation (inlet); D, Symptoms after artificial inoculation.

**Table 1.** Comparison of mycological characteristics of the present isolate obtained from Chinese chive (*Allium tuberosum* Roth.) and *Sclerotium rolfsii* as previously described

Characteristics		Present isolate	<i>S. rolfsii</i> [1]
Colony	Color	White	White
Hyphae	Diameter ( $\mu\text{m}$ )	4~8	4.5~9
	Clamp connection	Present	Present
Sclerotium	Shape	Globoid	Spherical
	Diameter (mm)	1~3	1~2
	Color	White to brown	Brown

No. GU567776). Phylogenetic analysis was performed using MEGA4 software using the neighbor-joining method and the Tajima-Nei distance model. Previously published ITS sequences of *S. rolfsii* strains were included for reference, and *Botryotinia fuckeliana* was used as the outgroup. In the phylogenetic tree (Fig. 2), the representative isolate was placed within a clade comprising reference isolates of *S. rolfsii*.

Based on the symptoms, mycological characteristics, ITS sequence analysis, and pathogenicity to the host plant,



**Fig. 2.** Phylogenetic tree using internal transcribed spacer sequences showing closest known relatives of *Sclerotium rolfsii*, including sclerotium rot fungus infecting *Allium tuberosum*. DNA sequences from the NCBI nucleotide database were aligned using ClustalW, and a phylogenetic tree was constructed using the neighbor-joining method and visualized with TreeView. Numbers above the branches indicate the bootstrap values. Bars indicate number of nucleotide substitutions per site. The present isolate infecting *A. tuberosum* is marked in bold.

this fungus was identified as *S. rolfssii* Saccardo [1]. Sclerotium rot in Chinese chive caused by *S. rolfssii* has not been previously reported in Korea [4]. Cultures of *S. rolfssii* were deposited in the Korean Agricultural Culture Collection (KACC 45155), National Academy of Agricultural Science, Rural Development Administration, Suwon. To our knowledge, this is the first report of sclerotium rot in Chinese chive caused by *Sclerotium rolfssii* in Korea.

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